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(54) Title: METHOD OF EVALUATING CHEMOTHERAPEUTIC AGENTS IN VIVO

(57) Abstract

The present invention relates to a method of screening chemotherapeutic agents *in vivo*. The method comprises implanting into a laboratory animal a biocompatible, semi-permeable encapsulation device containing a target cell-line, treating the laboratory animal with a test agent, then evaluating the target cells for reaction to the test agent.

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- 1 -

## METHOD OF EVALUATING CHEMOTHERAPEUTIC AGENTS IN VIVO

### Field of the Invention

The present invention relates to a method of screening  
5 chemotherapeutic agents in vivo using target cells grown in biocompatible,  
selectively permeable macrocapsules.

More particularly, the present invention involves a method for  
providing a retrievable sample of syngeneic, allogenic or xenogeneic cells into  
10 laboratory animals using cells or cell lines grown in selectively permeable hollow  
fibers or dialysis tubing. Following in vivo culture, with or without  
chemotherapeutic treatment, the cells are retrieved for in vitro evaluation of cell  
15 viability, cell density, cell growth potential, virus burden or other parameters  
appropriate for the test system.

15

### Background of the Invention

The desire for effective treatment against tumor growth and viral  
infection has created a need in the research and medical communities for a quick  
20 and reliable way to screen potential chemotherapeutic agents. That different types  
of tumor cell lines (e.g., renal, colon, pancreatic, etc.) react at different degrees to  
various chemotherapeutic agents, forces one to perform individual experiments  
usually screening one possible agent against one type of target cell or cell line.

25 For several years, this type of screening experiment was performed  
in vitro. For example, human tumor cells would be grown in semisolid media, a  
potential chemotherapeutic agent would be introduced into the media, and the  
viability of the cells after exposure would be measured. (See, Roper, P.R., and  
Drewinko, B., "Comparison of in vitro methods to determine drug-induced cell  
30 lethality", Cancer Res., vol. 36, pp. 2182-88 (1976)). However, the exposure of  
cells to drugs in vitro is highly artificial and restricts the use of drugs which  
require metabolic activation. It is doubtful whether such in vitro drug exposure  
can accurately reflect in vivo pharmacokinetics.

35 This led some researchers to develop ways in which to test possible

- 2 -

• chemotherapeutic agents in vivo. These models consist of implanting tumor cells into a laboratory animal, treating the animal with a possible chemotherapeutic agent, and then monitoring the animals to determine the effects of treatment on tumor growth. Several models were attempted, for example: (1) the  
5 subcutaneous tumor model which consists of surgically placing live tumor cells under the skin of a laboratory animal; (2) the subrenal tumor model, which measures the growth of tumor cells surgically implanted under the kidney capsule of laboratory animals; (3) the peritoneal model, in which the tumor cells are injected directly into the peritoneal cavity; and (4) the metastasis model, in which  
10 the primary tumor cells are injected into the foot of the laboratory animal.

These models are limited, however, because they only permit one type of tumor cell or cell line to be screened per experiment (per animal), which translates into the need for more test agent to screen several tumor lines.  
15 Furthermore, the animals need to be sacrificed to collect the tumor cells, and there is no way to control the effects of interaction between the tumor cells and the host laboratory animal.

Another model, which attempted to overcome these hurdles, is the  
20 agar diffusion chamber (Selby, P.J. et al., "Use of the Agar Diffusion Chamber for the Exposure of Human Tumor Cells to Drugs", Cancer Res., Vol. 42, pp. 4758-4762 (1982)). This method involves suspending tumor cells in agar, then introducing the suspended cells into a diffusion chamber. The diffusion chamber is then implanted into the peritoneal cavity of a laboratory animal, which is treated  
25 with a test agent. This method, however, suffered from the admitted drawbacks of being "labor-intensive and expensive" (Selby, p. 4761), as well as only permitting the implantation of one cell type or line because of the size of the diffusion chamber, and the presence of agar in the chamber.

Because of these drawbacks in the above in vivo models, a need still existed for a method of screening chemotherapeutic agents in vivo which was rapid, inexpensive, and which could be used to screen more than one cell line per experiment. The present invention satisfies this need by providing a method of  
30 screening test agents in vivo, involving the encapsulation of target cells in a device  
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- 3 -

comprised of a biocompatible semipermeable membrane, and implantation of the device (subcutaneously, intraperitoneally, or intra-organ) in a laboratory animal which is then treated with the test agent.

Encapsulation of cells has been described in the art for a variety of purposes. For example, Lacy, P.E. et al., "Maintenance of Normoglycemia in Diabetic Mice by Subcutaneous Xenografts of Encapsulated Islets", Science, vol. 254, pp. 1782-1784 (1991), describes the use of hollow fibers fabricated from an acrylic copolymer to encapsulate small numbers of rat islet cells that were then transplanted into mice. The encapsulation device prevented an immune response against the rat islet cells, and permitted diffusion of the bioactive compound insulin to maintain normoglycemia in mice. (See, also, Colton, C.K. et al., "Bioengineering in Development of the Hybrid Artificial Pancreas", J. Biomech. Eng., vol. 113, pp. 152-170 (1991)).

In addition, U.S. Pat. No. 4,324,683 and U.S. Pat. No. 4,352,883 to Franklin Lim both teach and describe encapsulation of biological material in spherical microcapsules. The microencapsulation techniques taught by these patents, however, have several limitations. First, their microencapsulation techniques generate aerosols which produce a biohazardous condition if the materials being encapsulated pose a health risk by aerosol transmission. Second, their microcapsules are extremely difficult to recover in toto from the host animal. Third, only the intraperitoneal implantation site is feasible if recovery of the implant is desired. And finally, it is difficult to recover the encapsulated cells without contamination by host cells. These limitations have been circumvented in the present invention.

Shockley, T.R. et al., "Penetration of Tumor Tissue by Antibodies and Other Immunoproteins", Ann. N.Y. Acad. Sci., pp. 367-382 (1991) discusses preliminary experiments to examine the feasibility of using the hollow fiber model for in vivo experiments. Shockley, however, does not teach or suggest using a hollow fiber system to test antiviral, antibacterial, and antiparasitic drugs in vivo. Shockley further does not teach or suggest that the hollow fiber system can be used to test a single drug against multiple tumor or cell lines. It has not previously been

- 4 -

- shown that the hollow fiber system may be used to test a single drug against multiple cell lines in a single mammal.

Finally, Allen, L.B. et al., "Novel Method for Evaluating Antiviral Drugs against Human Cytomegalovirus in Mice", *Antimicrobial Agents and Chemother.*, vol. 36, pp. 206-208 (1992), teaches a virus-host cell system in which human cytomegalovirus-infected human cells are entrapped in agarose plugs and cultivated *in vitro* or implanted into conventional mice. This system, however, is ineffective for encapsulation and implantation into mice of tumor cells or cells expressing a retrovirus such as HIV, because those cells will not replicate in the agarose concentrations of 1-1.5% taught by Allen.

All references cited and discussed above are herein incorporated by reference.

Despite the fact that microencapsulation and hollow fiber systems are known as described in the references cited above, the art still lacks a method for screening chemotherapeutic agents against one or more cell lines encapsulated in biocompatible, semi-permeable macroparticles and implanted in a single mammal.

Accordingly, an object of this invention is to provide a method wherein a cell, cell line or infectious agent is encapsulated in a biocompatible, semi-permeable device and implanted into a mammal, which is then treated with a chemotherapeutic agent intended to affect the encapsulated cell or infectious agent.

An additional object of this invention is to provide a method wherein multiple types of cells or infectious agents can be cultured simultaneously in a single mammal so that evaluating experimental chemotherapeutic agents does not require as much of said agent as has been historically required.

A further object of this invention is to provide a method wherein a single experimental animal can carry implants in multiple sites allowing simultaneous evaluation of a chemotherapeutic agent's capacity to reach various physiologic compartments (i.e., subcutaneous, intraperitoneal and intra-organ).

- 5 -

### Summary of the Invention

In accordance with these and other objects of the present invention, a method of evaluating a chemotherapeutic agent using cells grown in a biocompatible, semi-permeable device in vivo is provided. The present method 5 comprises providing a target cell or cells which are cultivated in vitro in medium appropriate for the cell line. Generally, a 10-20% serum concentration is recommended for cell lines which are routinely cultivated in serum-containing medium. Cells are collected, viability determined, and the cell density adjusted as needed for filling an encapsulation device.

10 After preparation, the cells are instilled into the encapsulation device. For the practice of the present invention, the encapsulation device is preferably a hollow fiber or dialysis tubing comprised of a biocompatible, semi-permeable material. After filling, the encapsulation device(s) is (are) implanted 15 directly into a laboratory animal, or the samples are incubated for a period of time to allow stabilization of cell growth before implantation. Each recipient animal can receive a single implant at a single site or can receive multiple implants at multiple sites.

20 The laboratory animal is then treated with the test chemotherapeutic agent on a dose and schedule appropriate for the agent being evaluated. The implanted samples are allowed to remain in the host animal for an appropriate period of time, sufficient to permit the test agent to affect the target cells. The 25 period of time varies for each test agent, and is preferably between 1 and 10 days. The implanted samples are then harvested for evaluation of the effects of the chemotherapeutic agent (called the endpoint).

This method provides a rapid and effective way in which to evaluate 30 the efficacy of chemotherapeutic agents against one or more target cells at one or more implant sites by comparing the endpoint results obtained in animals treated with the test agents to the results obtained in animals treated with a placebo (e.g., diluent controls).

- 6 -

Detailed Description of the Invention

For the practice of one embodiment of the present invention one must first select a target cell, cell line, or infectious agent. Such a target cell can include human tumor cell lines (e.g., melanomas, lung tumor lines, renal tumor lines, colon tumor lines, prostate tumor lines, ovarian tumor lines, breast tumor lines, central nervous system tumor lines, leukemic cell lines, etc.); human fibroblasts; human leukocytes; murine tumor cell lines (e.g., P388 murine leukemia); human tumor xenografts; fresh patient derived tumor tissue; human lymphoid cell lines; cultured primary cell lines; bacteria; virus-infected cell lines (e.g., human cell lines producing human immunodeficiency virus); yeast and fungi; as well as other cell lines, strains or tumors of mammalian species.

The target cells selected for cultivation in the encapsulation device are cultivated *in vitro* in a culture medium appropriate for the cell line.

Appropriate media known in the art include, but are not limited to: RPMI 1640; Eagles' minimal essential medium (MEM); Dulbecco's modified MEM, or others. For most cell lines the culture medium includes 10% fetal calf serum. Selected cell strains may require serum-free medium or other serum defined medium for culture. If fresh tumor tissue is used as the target, the cells are dispersed mechanically or enzymatically (using protein degrading enzyme, such as collagenase) before cultivation. The cells are cultured to maintain them in logarithmic growth as appropriate for each cell line. The cells are provided with fresh medium 24 hours prior to harvest for culture in hollow fibers.

One must then select an encapsulation device in which to cultivate the target cells *in vivo*. The encapsulation device can be any biocompatible, semi-permeable, implantable device. Preferred forms of macroencapsulation devices include selectively permeable hollow fibers or dialysis tubing. Suitable hollow fibers (HF) include, but are not limited to, those composed of polysulfone (PS), polyvinylidene fluoride (PVDF), cellulose acetate (CA-E), saponified cellulose ester (SCE) or polypropylene (PP). Suitable dialysis tubing (DT) includes, but is not limited to, that composed of regenerated cellulose (RC) or cellulose ester (CE).

Other biocompatible, semi-permeable materials can be used in a similar format,

- 7 -

depending upon their capacity to support cell growth. Following are the physical dimensions of some examples of macroencapsulation devices, not meant to limit the scope of possible devices to practice an embodiment of the present invention:

	<u>Material</u>	<u>Supplier</u>	<u>Internal Diameter</u>	<u>Mol. Wt. Cutoff</u>
5	PVDF HF	Spectrum	0.5 mm & 1.0 mm	300,000 & 500,000
	PS HF	Spectrum	0.5 mm & 1.0 mm	2,000; 10,000; 50,000; 100,000
10	CA-E HF	Spectrum	1.0 mm	unknown
	PP HF	Cellco	.33 mm	0.5 micron pores
15	SCE HF	Althin CD	.21-.215 mm	unknown
	CA HF	Althin CD	.22 mm	unknown
20	RC DT	Spectrum	variable	variable
	CE DT	Spectrum	variable	variable

Although any semi-permeable macrocapsule can be used which permits diffusion of the test agent into the device, a preferred embodiment of the present invention utilizes a device with a molecular weight cutoff of 50,000 daltons or higher.

After an appropriate macroencapsulation device is selected, the device must be prepared for loading with the target cells. PVDF, PS and CA-E hollow fibers are prepared by autoclaving 20 minutes to sterilize, flushing with 70% ethanol solution and incubating in 70% ethanol at room temperature for a period of about 24 hours or longer. The alcohol is removed with a sterile water rinse and then the fibers are filled with tissue culture medium containing 20% fetal calf serum (for cell lines which tolerate fetal calf serum) or with other proteins (collagen, fibrinogen, etc.) which can block possible protein binding sites.

Following incubation for about 12 hours or longer at 37°C, the fibers are ready to be loaded with cells or other viable culture material.

SCE and CA hollow fibers are potted together into bundles with epoxy, methacrylate glue, or other appropriate biocompatible adhesive. The fiber bundles are prepared by flushing with deionized water, then sterilized chemically.

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- 8 -

with 70% ethanol. The fiber bundles are flushed with sterile water to remove the alcohol and incubated in tissue culture medium containing 20% fetal calf serum or other appropriate proteins for about 12 hours or longer at 37°C, prior to loading with cells.

5           The RC and CE dialysis tubing are flushed with and boiled in deionized water to remove preservatives. The tubing is cut into 3-4 cm lengths and one end is sealed with a knot, methacrylate surgical glue, or other biocompatible adhesive, thereby forming "bags". The tubing is sterilized chemically with 70% ethanol, flushed with sterile water and incubated in tissue culture medium for about  
10           12 hours or longer at 37°C prior to loading with cells.

15           After appropriate preparation, the hollow fibers or dialysis tubing "bags" are ready to receive the target cells. First, the target cells or cell lines are collected and their viability determined. The cells are prepared at a cell density appropriate to maintain cellular growth in the hollow fibers. This density varies for each cell line, and must be established for each individually. As a general rule, most cells can be cultured at  $1-10 \times 10^6$  cells/ml of culture medium, although higher and lower ranges can be used.

20           For virus infected cells, acute infections can be performed immediately prior to transfer of the cells into hollow fibers. Alternately, chronically infected cells may be cultured. It is also possible to prepare co-cultivations by mixing virus-infected cells and uninfected target cells prior to loading them into hollow fibers. For neoplastic cells, no infection is performed, they are simply harvested and prepared at the appropriate cell density.  
25

30           After preparation, the target cells are instilled into the encapsulation device. The cells may be entrapped in agarose, extracellular matrix or other supportive materials prior to loading into the encapsulation device. Pretreatment of the encapsulation device with poly-L-lysine, collagen, fibrinogen and/or other attachment/pre-treatment agents prior to loading cells into the hollow fibers can also be used to enhance cell attachment or growth, or to prepare samples for specific tests (e.g., colony formation in agarose).

35           The inoculum (cells, bacteria, etc.) is transferred to sterile syringes

- 9 -

and the hollow fibers are filled using a needle of the appropriate gauge to fit into the hollow fiber or in the case of fiber bundles a piece of sterile tubing is used to connect the syringe to the bundle. The cells are flushed into the hollow fibers by applying gentle pressure on the syringe plunger. The ends on the PVDF, PS and  
5 CA-E fibers are heat sealed with smooth jawed needle holders or other appropriate devices that have been heated to 250°F or higher. Individual samples of PVDF, PS and CA-E for implantation are prepared from the filled hollow fibers by heat sealing the fibers at standard intervals along the tubing. Generally, for 1.0 mm diameter hollow fibers an interval length of 2 cm is used, while the 0.5 mm  
10 diameter hollow fibers are prepared in 3 cm lengths. These lengths are workable, however, any length can be used provided in vivo implantation is feasible (e.g., samples prepared for implantation in dogs are in lengths of 10-12 cm). The SCA and CA hollow fiber bundles are sealed on the ends with methacrylate surgical  
15 glue.

For dialysis tubing "bags", filling is accomplished by transferring the inoculum into the tubing with a micropipet or a needle/syringe. The dialysis tubing "bags" are closed with methacrylate surgical glue or other biocompatible sealants. While the length of these bags is dependent on the diameter of tubing  
20 being used, lengths of 2-4 cm have been demonstrated to be effective in mice.

After filling, the macrocapsules can be implanted directly into laboratory animals (mice, rats, dogs, etc.), or they can be incubated in vitro to allow stabilization of the culture and recovery from the effects of cell manipulation  
25 (i.e., trypsinization, centrifugation, cold shock, etc.) before implantation.

Macrocapsules are preferably implanted intraperitoneally or subcutaneously, or both. Samples are implanted into the intraperitoneal cavity by anesthetizing the mice with methoxyflurane (other anesthetics could be used) and making an incision through the skin over the ventral abdomen or, in a second approach, the skin over the lateral abdominal wall. The musculature of the abdomen is incised (1/2 cm or smaller incision) and the hollow fibers are passed through the incision and deposited in the abdominal cavity. For ventral incisions  
30 the musculature is closed with sterile suture (silk, cat gut, polyglycolic acid, etc.)

- 10 -

and the skin is closed in a second layer with suture, wound clips or surgical glue. For dorsal incisions, only the skin is closed (suture, wound clips, glue) as there is less risk of herniation at this site than ventrally. The mice are allowed to recover from anesthesia, with a heat source provided if recovery is slow.

5           For subcutaneous implants a skin incision is made over the dorsal thoracic region (nape of the neck) and the hollow fibers are passed caudally through the subcutaneous tissue. This passage is facilitated using a tumor-implantation trocar (11-16 gauge). The fibers are loaded into the distal end of the trocar which is then passed caudally through the skin incision. During withdrawal 10 of the trocar, the fiber(s) are deposited in the subcutaneous space using the trocar stylet to push the fiber out of the trocar. The skin incision is closed using suture, wound clips or surgical glue.

15           Generally, it is recommended that one implant the intraperitoneal fibers first, then roll the animal over and implant the subcutaneous fibers. However, the fibers can be implanted in any order.

20           Each recipient animal can receive a single implant (i.e., a single macrocapsule) at a single site (subcutaneous, intraperitoneal, or in a specific organ), or receive multiple implants at multiple sites (i.e., 3-6 (or more) macrocapsules implanted subcutaneously and intraperitoneally in each animal). Each macrocapsule can contain the same target cell, or each can contain a different cell or cell line, infectious agent or bacteria. In this way one can encapsulate one 25 or more cell lines in hollow fibers, implant each fiber intraperitoneally, subcutaneously, or intra-organ in the same animal, and treat the animal with an agent to determine its effect on cells cultured in the different physiologic compartments.

30           After implantation, the laboratory animals are treated with the test chemotherapeutic agent on a dose and schedule appropriate for the agent being evaluated. A single chemotherapeutic agent can be used during treatment, or two or more can be combined or given to the animal simultaneously to test the effect of the combination of compounds on the target cells. The chemotherapeutic agents 35 are administered by the intravenous, intraperitoneal, subcutaneous, oral and/or

- 11 -

• **percutaneous routes on any number of schedules.**

The implanted samples are allowed to remain in the host animal for an appropriate period of time, usually about 6 days. It is possible to use alternate schedules of either longer or shorter in vivo incubation periods to arrive at the 5 endpoint to determine activity of the agent on the target cells.

The implanted samples are then harvested at the appropriate time for in vitro evaluation of the effects of the chemotherapeutic agent(s). For antineoplastic compounds, the endpoint is determined by the quantity of viable cells 10 present in the macrocapsule which can be determined by tests known in the art, for example, MTT dye conversion assay, neutral red dye uptake, trypan blue staining, viable cell counts, the number of colonies formed in soft agar, the capacity of the cells to recover and replicate in vitro following in vivo exposure to the test agent(s), and others.

15 For evaluation of antiviral agents, the endpoint is determined by the number of viable cells present in the macrocapsule, the capacity of the cells to replicate, the presence of proteins, glycoproteins, nucleic acids or other products specifically associated with the infectious agent (e.g., reverse transcriptase, 20 nucleoprotein (HIV p24)), and/or the level of infectious material recovered from the macrocapsule.

25 Several experiments were run using the above described method to screen a variety of chemotherapeutic agents for effectiveness against tumor cell lines. The following examples illustrate one embodiment of the present invention, but should not be used to limit its scope.

Example 1

PVDF hollow fibers obtained from Spectrum, with an internal 30 diameter of 1.0 mm, and a molecular weight cutoff of 500,000 daltons were prepared as described above. These fibers were then instilled with one of the following tumor cell lines, cultivated in RPMI 1640 with 20% fetal calf serum: leukemia cell line HL60; central nervous system tumor cell line U251; ovarian tumor cell line Ovcar 3; lung tumor cell line H522 or H23; colon tumor cell line 35 SW620 or Colo 205; melanoma cell line LOX or SK-MEL-28; or renal tumor cell

- 12 -

line SN12K1 or SN12C. The fibers were then heat sealed with heated smooth jawed needle holders at intervals of two centimeters.

These macrocapsules were then implanted intraperitoneally into mice, which were then treated intraperitoneally with one of the following chemotherapeutic agents: methotrexate (NCI #740); vinblastine (NCI #49842); adriamycin (NCI #123127); L-PAM (NCI #241286); ellipticinium (NCI #155693); mitomycin C (NCI #26980); cyclophosphamide (NCI #26271); DTIC (NCI #45388); chlorambucil (NCI #3088); BCNU (NCI #409962); cis-platinum (NCI #119875); VP-16 (NCI #141540); actinomycin D (NCI #3053); fluorouracil (NCI #19893); or bleomycin (NCI #125066), or with a control. The control consisted of the diluent used to dissolve the chemotherapeutic agent.

After treatment with a specified dose and schedule as specified in Table 2, the mice were sacrificed and the tumor cell viability determined by MTT (3-[4,5-dimethylthiazol-2-y1]-2,5-diphenyltetrazolinum bromide) assay (Cancer Res., 51:1247 (1991)). To perform this assay, a small amount of MTT is added to a sample of cells which have been collected. MTT is bright yellow/gold in color, but is converted to MTT-formazan (dark purple color) by viable cells. The greater the amount of viable cells present, the more pronounced the color change. The color is then measured in a spectrophotometer at 540 nm, using empty hollow fibers as a blank. A cell viability measurement of the control macrocapsules is also measured, and from these measurements a percentage of treated tumor growth as compared to control growth is determined using the formula: %tumor growth in treated animals compared to control animals =  $100 \times (\text{MTT measurement for treated cells-blank}) / (\text{MTT measurement for control cells-blank})$ .

For the present experiments, a positive response in the hollow fiber assay was defined as a reduction in viable cells of 25% or more in the drug treated mice compared to the control mice. Therefore, if the above formula is used to determine the percentage of tumor cell growth in treated animals as compared to control animals, a value of 75 or less would indicate that the tumor cell line contained in the hollow fiber and implanted in an animal treated with a chemotherapeutic agent was negatively affected (cell growth inhibited compared to

- 13 -

control). Values of 76 or greater indicate that the tumor cells did not respond well to the tested agent.

The results for several experiments performed using PVDF hollow fiber encapsulated tumor cell lines, implanted intraperitoneally, are contained in 5 Table 1. The dosage and dosing schedules used in these experiments are listed in Table 2. The Q2D X 3 schedule indicates treatment with the indicated drug every two days (every other day) for a total of three infusions; QD x 5 indicates daily infusion for five days; Q8H x 2 indicates infusion every eight hours, twice a day.

10 Table 1 indicates that by using the method of the present invention, one can easily screen potential chemotherapeutic agents for effectiveness against tumor cell growth. Column one of Table 1 lists the agents screened; column two gives the National Cancer Institute number assigned to each agent (NCI#); column three gives the experiment number which corresponds to experiment numbers in 15 Table 2; and column four gives the dose administered in mg/kg/injection.

#### Example 2

In the same test animals of Example 1, PVDF hollow fibers containing the same tumor cell lines as in Example 1 above were simultaneously 20 implanted subcutaneously. These animals were tested using the same chemotherapeutic agents and dose schedules as in Example 1, or were treated with diluent (control). The percent growth of the subcutaneously implanted tumor cells in treated animals compared to control animals was determined. The results are 25 contained in Table 3.

#### Example 3

In other experiments, the tumor cell lines described in Example 1 30 were encapsulated in PS hollow fibers. The PS hollow fibers were obtained from Spectrum, and had an internal diameter of 0.5 mm and a molecular weight cutoff of 50,000 daltons. These fibers were heat sealed and separated into macrocapsules three centimeters in length. These PS encapsulated tumor cells were implanted intraperitoneally in mice, which were then treated intraperitoneally with one of the chemotherapeutic agents listed in Example 1, or were treated with diluent (control). 35 The dosage schedules for these experiments are contained in Table 2. The percent

- 14 -

growth of tumor cells in treated animals compared to control animals was determined. The results are contained in Table 4.

It has been shown that the method of the present invention is useful  
5 in evaluating the efficacy of chemotherapeutic agents against one or more targets  
(i.e. tumor types, viruses, bacterial strains, etc.) at one or more implant sites  
(subcutaneous, intraperitoneal) by comparing the endpoint results obtained in  
10 animals treated with the test agents to the results obtained in animals treated with  
placebo (e.g., diluent controls). The advantages of this system over the classical  
antitumor animal models includes one or more of the following: (1) the  
15 requirement for a smaller amount of test agent to assess preliminary in vivo  
efficacy, for example, to screen a drug dosed at 100 mg/kg the classical model  
would require a total of 160 mg of test agent, while using the method of the  
present invention would require 35 mg of test agent, (2) the rapid turn-around time  
20 (10 day assay), (3) the ability to test potential activity against multiple tumor types  
simultaneously in the same experimental animals, for example, implanting six  
macrocapsules each containing a different cell line, (4) a reduction in the number  
of experimental animals required for assessing efficacy (the classical model  
requires 30 animals while the present method uses 6), (5) the possibility of  
assessing multiple treatment schedules in a short time frame, (6) the ability to  
recover the entire implant, (7) the lack of contamination of the implanted cells by  
25 host cells, and (8) the ability to screen a test agent against target cells cultivated in  
various physiologic compartments in the same laboratory animal, for example,  
implanting macrocapsules containing target cells intraperitoneally, subcutaneously  
and intra-organ in the same animal and subjecting the animal to a test agent. These  
same advantages exist for assessing antiviral agents and have the added benefit of  
30 allowing short-term replication of viruses in susceptible cell lines in a host (i.e.,  
mouse, rat, dog, etc.) which does not normally support replication of the viruses.

Additionally, the present method can be used in transgenic animals to  
determine the effect of a transgene or protein product thereof on a target cell line.  
35 Or, encapsulated target cells can be implanted in humans for screening of test

- 15 -

agents. Further, by encapsulating target cells and screening them against a test agent in vivo, then harvesting the cells, re-cultivating them and re-screening them by repeating the present method, it is possible to assess the regrowth potential of cells surviving treatment and then determine their susceptibility to a second treatment with the same or alternate anticancer agents.

The invention has been described in detail with particular reference to a preferred embodiment thereof, but it will be understood that the invention is capable of other and different embodiments. As is readily apparent to those skilled in the art, variations and modifications can be affected within the spirit and scope of the invention. Accordingly, the foregoing disclosure and description are for illustrative purposes only, and do not in any way limit the invention, which is defined only by the claims.

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- 16 -

TABLE 1

PVDF Tubing: Fibers Implanted IP		Normalized MTT Reading: 100x(value-blank)/(control-blank)									
		Cell Type									
		Leuk.	CNS	Ovar.	nsLung	Colon		Melan.		Renal	
Drug:	NCI# exp# dose	HL60 U251	Over3	H522	H23	SW620	Colo205	LOX	Sk-Mel-28	Sn12K1	SN12C
Methotrexate	740 48	30	48	70						45	
Methotrexate	740 31	30								105	
Vinblastine	49842 48	0.75	-2	133						4	
Vinblastine	49842 22	2				36				72	
Vinblastine	49842 27	0.75				14				73	
Vinblastine	49842 37	0.75	37				35	16			
Adriamycin	123127 48	4	5	24						2	
Adriamycin	123127 31	4					15			109	
L-PAM	241286 48	6	-2							23	
L-PAM	241286 27	6								58	
L-PAM	241286 37	6								61	
Elliptinium	155693 48	25	25								38
Mitomycin C	26980 51	2.5	51		32	21		38			51
Mitomycin C	26980 64	1.67								32	
Mitomycin C	26980 31	2.5						1			60
Cyclophosphamide	26271 51	60	129		108						105
Cyclophosphamide	26271 31	60						83			105
											68

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TABLE 1 (cont'd.)

PVDF Tubing: Filters Implanted IP		Normalized MTT Reading: 100x (value-blank)/(control-blank)									
		Cell Type									
		Leuk.	CNS	Ovar.	nsLung		Colo		Melan.	Renal	
Drug:	NCL# exp# dose	HL60	U251	Ovcar3	H522	H23	SW620	Colo205	LOX	Sk-Mel-28	Sn12K1
DTIC	45388 51 250	146		103						85	
DTIC	45388 27 250						108			81	129
DTIC	45388 37 250			63				57	35		
Chlorambucil	3088 51 25	19		71						52	
Chlorambucil	3088 22 25					6				153	6
BCNU	409962 51 25	141					86	94			
BCNU	409962 31 10							71		96	60
Cis-Platinum	119875 51 3.5	32					56		67		
Cis-Platinum	119875 37 5			26				43	18		
Cis-Platinum	119875 27 5							56		69	65
VP-16	141540 22 50							75		132	34
Actinomycin D	3053 27 0.2							1		83	37
Actinomycin D	3053 37 0.2			4				20	1		
S-Fluorouracil	19893 27 40							167		83	88
S-Fluorouracil	19893 37 40			74				76	50		
Bleomycin	125066 31 10							82		100	113

- 18 -

TABLE 2

	<u>Expt #</u>	<u>NCI #</u>	<u>Dose</u>	<u>Schedule</u>
5	14	740	40mg/kg/inj	Q2D X 3, start Day 1
		26271	60mg/kg/inj	Q2D X 3, start Day 1
		26980	2.5mg/kg/inj	Q2D X 3, start Day 1
10	17	740	40mg/kg/inj	Q2D X 3, start Day 1
		26271	60mg/kg/inj	Q2D X 3, start Day 1
		26980	2.5mg/kg/inj	Q2D X 3, start Day 1
15	19	740	40mg/kg/inj	Q2D X 3, start Day 1
		26271	60mg/kg/inj	Q2D X 3, start Day 1
		26980	2.5mg/kg/inj	Q2D X 3, start Day 1
20	22	3088	25mg/kg/inj	Q2D X 3, start Day 1
		49842	2mg/kg/inj	Q2D X 3, start Day 1
		141540	50mg/kg/inj	Q2D X 3, start Day 1
25	27	3053	0.2mg/kg/inj	Q2D X 3, start Day 1
		241286	6mg/kg/inj	Q2D X 3, start Day 1
		19893	40mg/kg/inj	Q2D X 3, start Day 1
30	31	45388	250mg/kg/inj	Q2D X 3, start Day 1
		49842	0.75mg/kg/inj	Q2D X 3, start Day 1
		119875	5mg/kg/inj	Q2D X 3, start Day 1
35	37	740	30mg/kg/inj	Q2D X 3, start Day 1
		26271	60mg/kg/inj	Q2D X 3, start Day 1
		26980	2.5mg/kg/inj	Q2D X 3, start Day 1
44	44	123127	4mg/kg/inj	Q2D X 3, start Day 1
		125066	10mg/kg/inj	Q2D X 3, start Day 1
		409962	10mg/kg/inj	Q2D X 3, start Day 1
35	44	3053	0.2mg/kg/inj	Q2D X 3, start Day 1
		241286	6mg/kg/inj	Q2D X 3, start Day 1
		19893	40mg/kg/inj	Q2D X 3, start Day 1
35	44	45388	250mg/kg/inj	Q2D X 3, start Day 1
		49842	0.75mg/kg/inj	Q2D X 3, start Day 1
		119875	5mg/kg/inj	Q2D X 3, start Day 1

- 19 -

	123127	4mg/kg/inj	Q2D X 3, start Day 1
	125066	10mg/kg/inj	Q2D X 3, start Day 1
	409962	10mg/kg/inj	Q2D X 3, start Day 1
48	740	30mg/kg/inj	Q2D X 3, start Day 1
	3053	0.2mg/kg/inj	Q2D X 3, start Day 1
	49842	0.75mg/kg/inj	Q2D X 3, start Day 1
	123127	4mg/kg/inj	Q2D X 3, start Day 1
	241286	6mg/kg/inj	Q2D X 3, start Day 1
	155693	25mg/kg/inj	Q2D X 3, start Day 1
51	26980	2.5mg/kg/inj	Q2D X 3, start Day 1
	26271	60mg/kg/inj	Q2D X 3, start Day 1
	45388	250mg/kg/inj	Q2D X 3, start Day 1
	119875	3.5mg/kg/inj	Q2D X 3, start Day 1
	409962	10mg/kg/inj	Q2D X 3, start Day 1
	3088	25mg/kg/inj	Q2D X 3, start Day 1
64	26980	1mg/kg/inj	QD X 5, start Day 1
	26980	0.5mg/kg/inj	QD X 5, start Day 1
	26980	0.5mg/kg/inj	Q8H X 2, Days 1-5
	26980	1.67mg/kg/inj	Q2D X 3, start Day 1
	26980	0.835mg/kg/inj	Q2D X 3, start Day 1
	26980	5mg/kg/inj	QD X 1, Day 1

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TABLE 3

PVDF Tubing: Fibers Implanted SC		Normalized MTI Reading: 100x(white-blank)/(control-blank)							
Drug:	NCI#	expt#	dose	Cell Type				Melan.	Renal
				Leuk.	CNS	Ovar.	nasLung		
Methotrexate	740	48	30	22	91			65	
Methotrexate	740	31	30			91		81	49
Vinblastine	49842	48	0.75	48	87			95	
Vinblastine	49842	22	2			105		153	282
Vinblastine	49842	27	0.75			118		83	96
Vinblastine	49842	37	0.75		101		163	41	
Adriamycin	123127	48	4	88	110				54
Adriamycin	123127	31	4			82		59	103
L-PAM	241286	48	6	37			92		77
L-PAM	241286	27	6			131		121	109
L-PAM	241286	37	6		85			111	142
Ellipticinium	155693	48	25	99	88				98
Mitomycin C	26980	51	2.5	42	62	67	75	47	65
Mitomycin C	26980	64	1.67				48		
Mitomycin C	26980	31	2.5				23		
Cyclophosphamide	26271	51	60	102	95				120
Cyclophosphamide	26271	31	60				60		63

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TABLE 3 (cont'd.)

PVDF Tubing: Fibers Implanted SC		Normalized MTI Readings: 100x/(value-blank)/(control-blank)									
Drug:	NCI#	expt#	dose	Cell Type				Renal			
				Leuk.	CNS	Ovar.	Small	Colon	Melan.	Sk-Mel-28	Sk-Mel-28
DTIC	45388	51	250	73	65					76	
DTIC	45388	27	250				81			117	100
DTIC	45388	37	250		51			71	19		
Chlorambucil	3088	51	25	15	65					61	
Chlorambucil	3088	22	25				83			157	297
BCNU	409962	51	25	74			119	108			
BCNU	409962	31	10					86		66	51
Cis-Platinum	119875	51	3.5	39			103		80		
Cis-Platinum	119875	37	5	63				73	54		
Cis-Platinum	119875	27	5					27		137	83
VP-16	141540	22	50					88		189	205
Actinomycin D	3053	27	0.2					99		121	83
Actinomycin D	3053	37	0.2		93				127	80	
5-Fluorouracil	19893	27	40					82		113	123
5-Fluorouracil	19893	37	40		80				125	99	
Bleomycin	125066	31	10					89		64	78

- 21 -

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TABLE 4

PS Tubing: Fibers Implanted IP	Drug:	NCI#	exp#	dose	Normalized MTT Reading: 100x (value-Mean)/(control-Mean)						Renal	
					Leut.	CNS	Ovar.	melung	Colon	Melan.	Sk-Mel-28	
Methotrexate	740	48	30	42								41
Methotrexate	740	31	30						90			
Methotrexate	740	14	40						129			
Methotrexate	740	17	40						101			
Methotrexate	740	19	40						100			
Vinblastine	49842	48	0.75	2				-11				-2
Vinblastine	49842	27	0.75						153			
Vinblastine	49842	37	0.75	21					34	6		
Adriamycin	123127	48	4	-3				-7				-2
Adriamycin	123127	31	4						2			27
L-PAM	241286	48	6	-4					-6			35
L-PAM	241286	27	6						7			84
L-PAM	241286	37	6		55				28	14		
Ellipticinium	155693	48	25	130	1							42
Mitomycin C	26980	51	2.5	-5				3	-8			31
Mitomycin C	26980	64	1.67						0			
Mitomycin C	26980	31	2.5						-1			51

- 22 -

- 23 -

TABLE 4 (cont'd.)

PS Tubing: Fibers Implanted IP		Normalized MTT Rating: 100x (value-blank)/(control-blank)										
Drug:	NCI# exp# dose	Cell Type										Renal
		Leuk.	CNS	Ovar.	mLung	H522	H23	SW620	Colo205	LOX	Sk-Mel-28	Sv12K1
Mitomycin C	26980	14	2.5				2					
Mitomycin C	26980	17	2.5				4					
Mitomycin C	26980	19	2.5				2					
Cyclophosphamide	26271	51	60	64	125							110
Cyclophosphamide	26271	31	60				44					171
Cyclophosphamide	26271	14	60									53
Cyclophosphamide	26271	17	60									49
Cyclophosphamide	26271	19	60									8
DTIC	45388	51	250	90	248							70
DTIC	45388	37	250									90
Chlorambucil	3088	51	25	3	44							51
Chlorambucil	3088	22	25									55
BCNU	409962	51	25	46								112
BCNU	409962	31	10									212
Cis-Platinum	119875	51	3.5	-5								1224
Cis-Platinum	119875	37	5									46
												17
												7

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TABLE 4 (cont'd.)

PS Tubing: Fibers Implanted IP		Normalized MTT Reading: 100x(value-Blank)/(control-Blank)									
		Cell Type									
Drug:	NCI#	expt#	dose	Lent.	CNS	Ovar.	ns Lung	Colon	Melan.	Renal	
				HL60	U251	Ovar3	H522	HeLa	Sk-Mel-28	Sn12K1	Sn12C
Cis-Platinum	119875	27	5				33		66		
VP-16	141540	22	50				61		108	55	
Actinomycin D	3053	27	0.2				2		39		
Actinomycin D	3053	37	0.2	7				1	1		
5-Fluorouracil	19893	27	40				133			81	
5-Fluorouracil	19893	37	40			95		91	9		
Bleomycin	125066	31	10				53		92		105

- 25 -

We Claim:

1. A method of screening chemotherapeutic agents in vivo, comprising the steps of:
  - implanting at least one biocompatible, semi-permeable encapsulation device containing a group of target cells into a mammal, said device being maintained in vivo for a pre-selected period of time;
  - removing said device; and
  - evaluating said target cells.
- 10 2. The method of claim 1, wherein said mammal is treated with a chemotherapeutic agent to subject said biocompatible, semi-permeable encapsulation device with said agent.
- 15 3. The method of claim 1, wherein a multiplicity of encapsulation devices are implanted.
4. The method of claim 3, wherein at least one encapsulation device is implanted subcutaneously and at least one encapsulation device is implanted intraperitoneally.
- 20 5. The method of claim 4, wherein said encapsulation devices are each instilled with the same target cells.
6. The method of claim 4, wherein said encapsulation devices are each instilled with different target cells.
- 25 7. The method of claim 1 wherein said target cells are selected from the group consisting of tumor cells, fibroblasts, leukocytes, lymphoid cells, cultured primary cells, bacteria, virus-infected cells, yeast and fungi.
- 30 8. The method of claim 7 wherein said tumor cells are selected from the group consisting of melanomas, lung tumor lines, prostate tumor lines, ovarian tumor lines, breast tumor lines, renal tumor lines, colon tumor lines, central nervous system tumor lines, leukemic or lymphoma cell lines, tumor xenografts and fresh patient derived tumor tissue.
- 35 9. The method of claim 1 wherein said biocompatible, semi-permeable encapsulation device is selected from the group consisting of hollow fiber and dialysis tubing.

- 26 -

10. The method of claim 9 wherein said hollow fiber is comprised of a material selected from the group consisting of polysulfone, polyvinylidene fluoride, cellulose acetate, saponified cellulose ester, polypropylene, polyamide and polyvinyl chloride acrylic co-polymer.

5 11. The method of claim 9 wherein said dialysis tubing is comprised of a material selected from the group consisting of regenerated cellulose and cellulose ester.

10 12. The method of claim 1 wherein said mammal is selected from the group consisting of a mouse, rat, hamster, mini-pig, rabbit, cat and dog.

13. The method of claim 1 wherein said implanting step comprises subcutaneous, intraperitoneal, or intra-organ implantation.

15 14. The method of claim 1, wherein said biocompatible, semi-permeable encapsulation device is comprised of a material permeable to compounds with a molecular weight of between about 50,000 daltons and about 500,000 daltons.

20 15. The method of claim 9 wherein said biocompatible, semi-permeable encapsulation device is comprised of a material permeable to compounds with a molecular weight of between about 50,000 daltons and about 500,000 daltons.

16. The method of claim 1 wherein said evaluating step is performed in vitro

25 17. The method of claim 1 wherein said evaluating step is performed in vivo.

18. The method of claim 7, wherein said target cell is a retrovirus-infected cell.

30 19. The method of claim 18, wherein said retrovirus-infected cell contains the human immunodeficiency virus.

20. The method of claim 1 wherein said target cell is infected with a virus incapable of replication in said mammal.

## INTERNATIONAL SEARCH REPORT

Intern'l Application No  
PCT/US 94/04443

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 A61K49/00 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA,A,2 065 662 (RHODE ISLAND HOSPITAL) 3 April 1993 see the whole document ----	1-10, 12-17
X	EP,A,0 218 400 (DAMON BIOTECH) 15 April 1987 see the whole document ----	1-8, 12-14, 16, 17
X	ANTIVIRAL RESEARCH, vol.10, no.4,5, 1988 pages 179 - 191 X.Q. LI ET AL. 'A NEW IN VIVO ANTI-VIRAL ASSAY USING MICROENCAPSULATED INFECTED CELL CULTURES' see the whole document ----	1-3,5,7, 12-14, 16-20
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

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## INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/04443

## C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER RESEARCH, vol. 47, no. 21, 1987 pages 5739 - 5747 E. GORELIK ET AL. 'MICROENCAPSULATED TUMOR ASSAY: NEW SHORT-TERM ASSAY FOR IN VIVO EVALUATION OF THE EFFECTS OF ANTICANCER DRUGS ON HUMAN TUMOR CELL LINES' see the whole document ---	1-3, 5-8, 12-14, 16, 17
X	CHINESE MEDICAL JOURNAL (TAIPEI), vol. 51, no. 3, March 1993 pages 166 - 175 J.M. HWANG ET AL. 'A NEW IN VIVO ASSAY OF THE REACTIONS OF MICROENCAPSULATED HUMAN TUMOR CELLS TO CHEMOTHERAPEUTIC DRUGS' see the whole document ---	1-3, 5-8, 12-14, 16, 17
X	PROCEEDINGS OF THE NATIONAL SCIENCE COUNCIL, REPUBLIC OF CHINA, vol. 12, no. 4, 1988 pages 252 - 261 C.F. CHEN ET AL. 'MICROENCAPSULATED OF TUMOR CELLS AND ASSAY FOR SELECTING ANTICANCER DRUGS' see the whole document ---	1-3, 5-8, 12-14, 16, 17
X	JOURNAL OF THE NATIONAL CANCER INSTITUTE, vol. 82, no. 22, 1990 pages 1761 - 1765 J. MCMAHON ET AL. 'FEASIBILITY OF CELLULAR MICROENCAPSULATION TECHNOLOGY FOR EVALUATION OF ANTI-HUMAN IMMUNODEFICIENCY VIRUS DRUGS IN VIVO' see the whole document ---	1-3, 5, 7, 12-14, 16-20
A	FR,A,2 073 007 (WORTHINGTON BIOCHEMICAL CORPORATION) 24 September 1971 see page 2, line 30 - page 3, line 30 see page 5, line 35 - page 6, line 4; claims 1,7 ---	11
A	WO,A,89 04655 (BROWN UNIVERSITY RESEARCH) 1 June 1989 see abstract; claims 1-3 ---	9, 10
A	WO,A,91 15245 (THE UNITED STATES OF AMERICA) 17 October 1991 see abstract; claims 1-19, 25 -----	1-20

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/US 94/04443

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
CA-A-2065662	03-04-93	JP-A-	6125912	10-05-94
EP-A-0218400	15-04-87	AU-B- AU-A- CA-A- JP-A-	571144 6279786 1265443 62122598	31-03-88 19-03-87 06-02-90 03-06-87
FR-A-2073007	24-09-71	BE-A- DE-A- NL-A-	759911 2060121 7017848	17-05-71 16-06-71 08-06-71
WO-A-8904655	01-06-89	US-A- AU-A- DE-A- EP-A,B JP-T- US-A- US-A- US-A- US-A- US-A- US-A-	4892538 2718488 3878918 0388428 3502534 5158881 5106627 5283187 5182111 5284761 5156844	09-01-90 14-06-89 08-04-93 26-09-90 13-06-91 27-10-92 21-04-92 01-02-94 26-01-93 08-02-94 20-10-92
WO-A-9115245	17-10-91	AU-A-	7656991	30-10-91

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